

Application No. 10/669,781  
Reply to Office Action of September 12, 2005

AMENDMENTS TO THE SPECIFICATION

Please replace Table 4 at the top of page 21 of the specification with the following Table 4:

Table 4: Peptides obtained by N-terminal amino acid sequencing

MW (det.)	MW (calc.)	amino acid sequence
		LSDPYHFTVAAAETEPVDTAGDAA * (SEQ. ID. No.: 3)
		LSDPYHFTVAAAETEPVDTAGDAADDPAILD (SEQ. ID. No.: 4)
932	932.1	YYAMVTGK (SEQ. ID. No.: 5)
1271.4	1271.3	EGEFEQYELK (SEQ. ID. No.: 6)
1050.3	1050.2	MLHSYNTGK (SEQ. ID. No.: 7)
798.9	798.9	IVPWER (SEQ. ID. No.: 8)
2951.2	2948.4	IVPWERIADQIGFRPLANEQVDPRK (SEQ. ID. No.: 9)
3467		NGTLQSMTDPDHPIATAINEVYGF TLWHSQ (SEQ. ID. No.: 10)
5450.2		YVADFRITDG PETDGT SDDDGII (SEQ. ID. No.: 11)
775.7	775.8	LTDRSGK (SEQ. ID. No.: 12)
1317.9	1317.4	VDIAASNRSEGK (SEQ. ID. No.: 13)
2167.4	2167.4	IADQIGFRPLANEQVDPRK (SEQ. ID. No.: 14)
720.7	720.8	ANQNFK (SEQ. ID. No.: 15)
619.6	619.7	VRAFK (SEQ. ID. No.: 16)
		LNNVDIRYDFP (SEQ. ID. No.: 17)
1779.4	1778	LNNVDIRYDFPLNGK (SEQ. ID. No.: 18)
1236.3	1236.4	NTIEIY AIDGK (SEQ. ID. No.: 19)
1137.4	1137.3	SGLVVYSLDGK (SEQ. ID. No.: 20)
		FSAEPDGGSNGTVIDRADGRHL (SEQ. ID. No.: 21)

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Please replace Table 5 at the top of page 22 of the specification with the following Table 5:

Table 5: PCR primers giving only one fragment each under optimal conditions

number	oligonucleotide sequence
6465	TCIGATCCITATCATTTCACIGT ( <u>SEQ. ID. No.: 22</u> )
6467	AG(C/A)GGAAAATCATAIC(C/T) (G/A)ATATC ( <u>SEQ. ID. No.: 23</u> )
6469	CTTCIGAIC(G/T) (G/A)TTIGAIGCIGC ( <u>SEQ. ID. No.: 24</u> )
6470	TGATCIGC(G/A)ATIC(G/T)TTCCCA ( <u>SEQ. ID. No.: 25</u> )
6471	GC(G/A)AT(C/A)GGATGATC(C/A)GGATC ( <u>SEQ. ID. No.: 26</u> )
6472	TTCATA(C/T)TGTTCAAATTCCICC ( <u>SEQ. ID. No.: 27</u> )
6473	TTICCGT(G/A)TTATAIGAATGIA- (G/A)CAT ( <u>SEQ. ID. No.: 28</u> )
6474	CCATC(G/A)ATIGCATA(G/A)ATTTC ( <u>SEQ. ID. No.: 29</u> )
6541	TTTAAA(G/A)TT(C/T)TG(G/A)TTIGC ( <u>SEQ. ID. No.: 30</u> )
6544	TTTICCGTIAACCATIGC ( <u>SEQ. ID. No.: 31</u> )

Please replace the paragraph on page 23 under the heading "Southern blot analysis of phytase of the phytase gene" with the following paragraph:

Genomic DNA was isolated from *B. subtilis* B 13, as described in Sambrook et. al. (supra, 1989). Restriction enzymes used were those of Boehringer-Mannheim. *B. subtilis* B 13 DNA was partially digested with EcoRI and the fragments were separated on agarose gel. Separated fragments were Southern-Blotted to nylon membrane. Nylon membrane was Southern-Hybridized with 32P-labelled N-terminal oligonucleotide probe,  
GA(C/T)CC(G/A/T)TA(C/T)CA(C/T)TT(C/T)AC(G/A/T)GTNAA(C/T)GC  
(G/A/T)GC(G/A/T)GC(G/A/T)GAAAC (SEQ. ID. No.: 32), in order to determine the approximate size of the fragment containing the putative phytase gene. Southern-Hybridisation

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showed two bands of approximately 1700 bp and 1000 bp consistent with the structure of the gene given in FIG. 9.

Please replace the following 4 paragraphs beginning after “5’ primer for both pQE-30 and pQE-60 constructs:” and ending before “Said constructs were then transformed into the expression host...” with the following 4 paragraphs:

5' primer for both pQE-30 and pQE-60 constructs:

SEQ. ID. No.: 33

GTTTCTCAATTGAAGGAGGAATTTAATGCTGTCCGATCCTTATCATTTAC  
Mfe I RBS Met Leu Ser Asp Pro Tyr His Phe

3' primer for pQE-30 construct:

SEQ. ID. No. 35 AATAAGTCGACGTACGACCGGATTCCGGCTGTGCT  
Sal I

The 3' primer used for the pQE-60 construct encoded the C-terminus of the protein (without stop codon) followed by a Bgl II cloning site. The vector sequence provides the nucleotides encoding a histidine tag to facilitate purification of the expressed protein. The PCR product was cloned into pQE-60 digested with Eco RI/Bgl II. The enzyme expressed from this construct can be purified from the cell lysate using Ni-NTA resin according to the manufacturer's instructions (Qiagen).

### 3' primer for pQE-60 construct:

SEQ. ID. No.: 36 AATAAAGATCTTTCCGCTTCTGTCGGTCAGTT  
Bgl II

Please delete the previously filed "Sequence Listing" and replace it with the attached "Sequence Listing".